THE MEIOSTAGMINE REACTION: A CRITICAL REVIEW OF THE LITERATURE, AND A PERSONAL EXPERIENCE WITH THE METHOD.

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A laboratory reaction for the diagnosis of malignant disease has been a long-felt want. The subject has interested many workers and numerous different procedures have been elaborated with this end in view. Among these the isohemolytic index has found much favor and is said to give fairly constant results in a large percentage of sera derived from cancer subjects. The glycyltryptophan reaction for gastric carcinoma is another test upon which a large amount of experimentation is being done.

During the last year certain Italian workers, notably, Ascoli and his pupils, have presented a serological reaction based upon certain principles laid down by Ehrlich and also upon some laws of physical chemistry, by means of which they seem able to identify antibodies in the serum of the individual tested, when a specific antigen is employed.

Their procedure consists roughly of extracting the essential principles of the antigen, specific organism or diseased tissue being used, as the case may be, and adding this extract to the serum of the patient to be tested. The surface tension of the mixture is then ascertained. This is measured by the number of drops contained in a certain amount of the fluid, estimated with an instrument of precision, known as the stalagmometer of Traube.*

The mixture is then allowed to incubate at body temperature, and at the end of a definite time the surface tension is again estimated. Should the surface tension be decreased, the number of drops in the same amount of fluid will be increased, that is, the drops have become smaller. In other words, a chemophysical reaction will have occurred.

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* The stalagmometer of Traube is merely a very finely and elaborately graduated pipette containing a central bulbous reservoir so as to increase its total capacity to about 8 c.c. The lower, or dropping end of the instrument narrows down to a capillary bore which ends in a flattened ground base about 7 mm. in diameter, on which the solution to be tested accumulates until the drop finally falls, thus insuring uniformity in the size of the drops. The instrument is so graduated that a fraction of a drop can be estimated, and is calibrated so as to contain, when full, a definite number of drops of distilled water at 15° C. The stalagmometer of Traube can be bought from Fritz Kohler, of Leipsic, or C. Gerhardt, of Bonn.

Ascoli claims that if an antigen is incubated with a serum containing specific antibodies this lowering of surface tension will almost always occur, whereas in the absence of a specific antibody the surface tension remains unchanged. He refers to this phenomenon as the meiostagmine reaction (*meion*, small, and *stasso*, drop), because of the fact that the drops have become smaller. He does not attempt to explain the reaction, but claims for it a high degree of specificity. He refers to the specific body in the serum as meiostagmine. Meiostagmine is evidently a body of the first order. It does not need to be activated by another body, as, for example, a complement, although the presence of a complement does not interfere with it. It is not very thermolabile, since it can be heated as high as 56° C. without destroying its activity. The specific bodies in the antigen seem to be lipoids; they are extractable with methyl alcohol and with ether. Nothing more than this is known.

Meiostagmines, according to Ascoli and his pupils, occur in the blood in many diseases. They are present when the body has harbored certain bacteria, such as the typhoid or tubercle bacillus. In the case of the typhoid bacillus their presence is of greater diagnostic worth than that of the agglutinins which form the basis of the Widal reaction, for not only do they make the the diagnosis of typhoid fever certain, but the meiostagmines show no group tendencies, as is true with the agglutinins. Thus, by employing specific antigens of the Eberth bacillus and the Bacillus paratyphosus A or B, these diseases can be clearly differentiated.

In the case of patients infected with the tubercle bacillus, their presence and identification make the diagnosis certain and easy. Experimentally, laboratory animals were infected with human, bovine, and avian types of this bacillus, and in a very few days the presence of human, bovine, and avian tubercle meiostagmines could be made out by the presentation of their respective antigens. Accordingly, this is claimed to be a most accurate way of identification of the various types of the tubercle bacillus.

Following along these same lines, specific meiostagmines were identified in the serum of animals immunized against various substances, such as Witte's peptone, horse serum, etc.

In the case of syphilis, the diagnosis is even more accurate than that hitherto obtained by various complement fixation and deviation tests, such as the Wassermann test, inasmuch as cases of lepra that reacted positively to Wassermann's syphilis test were negative when tested for syphilitic meiostagmines.

Cases of ankylostomiasis and echinococcus showed positive results against their antigens.

In the case of patients suffering from malignant new growths, such as sarcoma or carcinoma, brilliant results were obtained in the identification of specific meiostagmines. In these cases the investigators were unable to separate carcinomatous from sarcomatous meiostagmines, the serum reacting interchangeably with antigen made of either type of malignant neoplasm. Benign growths, such as fibroma and lipoma, gave negative results against malignant tumor antigens.

Lastly, exudates formed in the pleural, peritoneal, and pericardial cavities in tuberculous and malignant disease when tested against their corresponding antigens gave positive results, the blood serum of the same patients showing no discrepancies in a parallel series of experiments.

These remarkable results show that the reaction has a wide scope of usefulness, inasmuch as it is of service in the diagnosis of many conditions, in some of which the etiological factor is known, in others of which we are at present only upon the threshold of a knowledge of the pathology, as in the case of malignant disease.

This much we will say in a general way as to the rationale and scope of the meiostagmine reaction as presented by Ascoli and his pupils before proceeding to a more detailed analysis of the work reported by them during the last year.

On January 11, 1910, Ascoli's[1] first article on the meiostagmine reaction appeared. He reported a series of experiments in which he tested the sera of typhoid patients against an antigen derived from typhoid bacilli and obtained diagnostic results. His technique was as follows: (*a*) 1 c.c. of serum was obtained in the usual manner, and without inactivation, diluted with 9 volumes of 0.85 per cent. sodium chloride solution. (*b*) The antigen was prepared after the method of Neisser and Shiga,[2] which was as follows: Typhoid bacilli were grown at 37° C. on agar for twenty-four hours, scraped off and emulsified thoroughly in normal saline. This emulsion was then heated in a water bath, kept between 55° and 60° C., for one hour. The emulsion was then reincubated for twenty-four hours at 37° C., and shaken several times during this period. It was then filtered through a Berkefeld porcelain filter and the filtrate used as antigen. Various dilutions of antigens were tested, ranging between 1 to 100 and 1 to 100,000, normal saline being used as diluent. (*c*) Method of mixing: 9 c.c. of diluted serum was added to 1 c.c. of the various dilutions of antigen and incubated at 37° C. for two hours. (*d*) Method of counting: The number of drops contained in a definite amount of this mixture was ascertained by means of Traube's[3] stalagmometer before and after incubation, the latter counting being done after the fluid had regained room temperature.

His results showed that when serum and 1 per cent. antigen were employed as above described there was an increase of 3.2 drops after incubation as compared with the count before incubation, providing a specific serum and antigen had been employed. Greater dilutions of antigen gave less than 2 drops' increase, while sodium chloride as antigen gave only 1 drop difference. A control with normal serum and typhoid extract gave an increase up to 1 drop.

He concludes from the above that a difference of 2 or more drops warrants a diagnosis of typhoid fever.

In attempting to arrive at a conclusion as to the nature of the specific endobody in the typhoid bacillus that was playing an active role in the reaction, he tried variously modified antigens and found: (1) That the reaction occurred with an alcoholic extract of the evaporated filtrate of the Neisser-Shiga emulsion. (2) He now added alcohol to the Neisser-Shiga salt solution antigen, and a precipitate occurred. This was filtered and the filtrate saved. The precipitate was extracted in alcohol and the extract massed with the above-mentioned filtrate. This combination was also active as antigen, but was not as potent as the first. (3) A watery extract of the precipitate mentioned in No. 2 was not potent as antigen.

The next contribution to the subject of the meiostagmine reaction appeared later during the same month by Izar,[4] a co-worker of Ascoli, and discussed the reaction in syphilis. Following Ascoli's technique, he used an extract of 5 grams of dried powdered syphilitic fetal spleen in 50 c.c. of absolute alcohol. This was stirred thoroughly and put into the thermostat for two hours and shaken repeatedly. It was then filtered and the filtrate concentrated to 10 c.c. and diluted as required. Sera were diluted 1 to 20 to 1 to 200, and 9 c.c. was used. Antigen was diluted 1 to 25 to 1 to 100,000, and 1 c.c. was used. It seems immaterial to the end result which strength antigen is used.

His known syphilitic cases gave 2 to 5 drops increase after incubation, whereas the same sera without antigen never showed more than 1 drop increase. Non-syphilitic cases were negative with syphilitic antigen. Two lepra cases with positive Wassermann reactions were negative with this reaction, using syphilitic antigen.

In studying the various organs that would give an antigen for this reaction, he found that alcoholic extract of guinea-pig heart (Landsteiner), alcoholic extract of normal human liver, and artificial extract of Sachs-Bordoni, all gave negative results, and that alcoholic extract of syphilitic liver gave positive results.

During the next month there appeared a contribution by Ascoli and Izar[5] on the meiostagmine reaction in cases of malignant tumors. The antigen used was prepared from rat sarcoma. The tumor was cut up finely and rubbed to a mushy consistency, extracted with 95 per cent. alcohol at 37° C. for twenty-four hours. The alcohol was then decanted and the process of extraction repeated twice more. The sediment was dried at 50° C. and extracted with warm ether three or four times in twenty-four hours. The ether was decanted and the sediment again extracted, with alcohol, until the fluid was colorless. All the decants were then massed and dried at 50° C. From the residue a saturated ether extract was made.

Before using it the antigen was diluted 1 to 10 to 1 to 500 with 0.85 per cent. saline solution. Sera to be tested were diluted 1 to 20

The serum of rats with sarcoma gave 4½ to 8 drops increase, whereas normal rat serum gave at most 1½ drops increase. In 62 cases of malignant newgrowth he obtained 58 positive reactions. Of 48 controls, all were negative. With an antigen derived from human carcinoma he found an increase of from 4 to 8 drops, but had to use antigen in dilutions of 1 to 10,000, as lower dilutions, strange to say, gave positive reactions with normal sera. The number of cases thus tested is not mentioned.

The next publication appeared March 12, 1910, by Izar and Usuelli.[6] This is a more extensive article, dealing with many points of laboratory technique which he considers necessary in order to obtain results. He makes the following statements: (1) The serum of the patient should be diluted with 0.85 per cent. salt solution. (2) First count the diluted serum alone, then add the antigen and incubate for two hours at 37° C. or for one hour at 50° C. (3) Remove and count the mixture, but only after it has been thoroughly cooled to room temperature. (4) It is imperative to determine the proper strength of antigen. (5) Antigen dilutions should be thoroughly emulsified. This is best done by placing the spirituous antigen in a tube and then adding the diluent all at once and shaking. (6) An increase of 2 or more drops is a positive reaction. (7) Sera may be kept a long time (not indefinitely however) in a cool place without much deterioration. (8) Alcoholic extract of guinea-pig heart and normal human liver, declared in a previous article to be worthless as a syphilitic antigen, he has found to be available. (9) Human sera of malignant disease and of mice and rats infected with carcinoma and sarcoma were tested against all three varieties of antigen (human malignant tumor, rodent carcinoma, and rodent sarcoma antigens), and found to react with all, but better when the homologous antigen was used.

In cases tested he obtained the following results: Ninety cases of syphilis gave 67 positive reactions; 18 cases of doubtful syphilis gave 8 positive reactions; 104 cases not infected with syphilis gave only 2 positive results against syphilitic antigen.

The next publication was by Izar,[7] in which he discussed the reaction in typhoid, tuberculosis, echinococcus disease and ankylostomiasis. Here a slight variation from the serum technique is mentioned, 15 to 20 drops of blood in distilled water being used instead of serum. The incubation period is also modified, leaving it to the discretion of the experimenter whether he shall incubate for two hours at 37° C., or one hour at 50° C., or two hours between 30° and 50° C., in the last case replacing the fluid evaporated with distilled water. Stock antigen, he says, can be kept long periods of time, but not indefinitely. Diluted antigen, however, will last hardly forty-eight hours.

In testing tuberculous cases, he found that of 40 cases of advanced tuberculosis, 39 were positive, whereas the controls were universally negative. In typhoid fever cases, 9 cases with positive Widal reactions, were all positive. In ankylostoma, of 6 cases, all were positive. In 11 cases of echinococcus disease all were positive.

Shortly after this Ascoli and Izar[8] reported upon the meiostagmine reaction in sera obtained from animals immunized against Witte's peptone, gelatin, and horse serum, using these substances respectively as antigens, and found the reactions of their sera positive and specific.

About the same time D'Este[9] reported from the surgical clinic of Professor Pausini, at Pavia, on the meiostagmine reaction in surgical conditions. In 15 cases of surgical tuberculosis where the diagnosis was controlled by operation his tests showed 1 to $2\frac{1}{2}$ drops increase. In 4 cases of pulmonary tuberculosis, all of which showed tubercle bacilli in the sputum, he obtained $1\frac{3}{10}$ to 3 drops increase. In testing 11 normal sera against tubercle bacillus antigen, he obtained not more than $1\frac{1}{10}$ drops increase.

In working with the sera of cases of malignant disease he found that in 12 cases (11 of which were operated upon) he obtained 1 to 3 drops increase. In 10 non-malignant neoplasms (8 of which were operated upon) he obtained negative results in all against malignant tumor antigen.

On May 24, 1910, Micheli and Cattoretti,[11] pupils of Ascoli, added the following data to the reports of the meiostagmine reaction: (1) The active elements of tumors employed in the reaction as antigen are lipoids and are thermolabile. (2) Not every specimen of malignant tumor will give extractile bodies that can be used as reagents. (3) Every antigen should be titrated so that one would be using the weakest solution that would produce 1 to $1\frac{1}{2}$ drops increase against a normal serum. (4) Antigen in stronger concentration than the above is meiostagmine positive with normal sera. (5) The active elements in the sera of tumor cases are different from all other previously known antibodies in that they are relatively thermostabile and have a peculiar reaction to ether as a reagent.

Two months later, Verson,[13] of Turin University, reported on a series of cases, verifying some of Ascoli's results, though not having quite as high a percentage of positive findings. Ten of 18 malignant tumors gave positive tests; 6 non-malignant cases were negative.

In testing sera derived from 3 cases of malignant disease against an antigen made from colloid goitre, he obtained a positive meiostagmine reaction in all 3 cases, whereas, of 2 goitre patients, only 1 gave a positive reaction. Normal sera were negative against this antigen. Extract of tuberculous lymph nodes was not satisfactory as anitgen against tuberculous cases.

In August, 1910, Stabilini,[14] from Pansini's clinic, working along the same lines as D'Este, reports: In 32 cases of malignant tumors, 10 of which had only clinical diagnoses, he found $2\frac{1}{10}$ to 3 drops increase. One case of carcinoma mammæ gave 7 drops increase, while 1 case of carcinoma ventriculi, proved microscopically, gave an increase of but $\frac{7}{10}$ drop. One case clinically diagnosticated as sarcoma of the thigh had only $1\frac{4}{10}$ drops increase. In 27 cases of non-malignant tumors and other conditions the results never showed an increase of more than $1\frac{1}{10}$ drops. He considers $1\frac{1}{2}$ to 2 drops increase as only suspicious.

At the same time, Gasharrini,[15] a pupil of Izar, studied the reaction in experimental tuberculosis and found that the sera of rabbits and guinea-pigs infected with human, bovine, and avian types of tubercle bacilli were meiostagmine positive against their homologous antigens only. The sera gave positive reactions four to five days after injection. He considers the test accurate enough to be used in differentiating the various types of tubercle bacilli.

Vigano,[16] a pupil of Ascoli, reported on the reaction in 6 typhoid cases. He obtained positive reactions in all with typhoid antigen, but negative readings with paratyphoid A and B antigens.

Gasharrini,[17] from Ascoli's laboratory, reported upon results obtained in testing various serous exudates for meiostagmines. In all, 25 fluids were tested: 4 pleural tuberculosis fluids and 2 peritoneal tuberculosis fluids were positive with tubercle antigen; 4 pleural exudates, 3 peritoneal exudates, 1 hydrothorax, 1 peritoneal transudate, and 1 pericardial fluid, all from cases of malignant disease, gave positive reactions against malignant tumor antigen; 5 idiopathic pleurisies not proved to be tuberculous were negative against tubercle antigen, while 4 exudates from non-malignant cases were negative against tumor antigen. In tests made upon the blood of all but 4 of the 25 cases, no discrepancies occurred when compared with the results upon the exudates.

The above historical review covers ten months, during which time the results reported were universally satisfactory. At this point, in an article published October 11, 1910, Ascoli and Izar[18] advocate certain changes in the technique of the reaction. They advocate a change in the preparation of antigen, which is indeed much simpler than that previously employed. They cut the tumor into fine pieces, dry in vacuo, pulverize the tumor, and treat 0.5 gram of the powder with 25 c.c. of methyl alcohol, at 50° C., in a closed vessel, for twenty-four hours, shaking from time to time. Filter warm, allow the filtrate to cool, and then filter again (Schleicher and Schull 590 filter paper). Titrate. The titer, they say, lies mostly between 1 to 100 and 1 to 200, that is, 1 c.c. of a 1 to 100 or 1 to 200 watery emulsion of this methyl alcohol antigen should be used to 9 c.c. of serum diluted 1 to 20 with 0.85 per cent. sodium chloride. The antigen should never be shaken. Always have a positive and a negative control serum. The best positive control is a mildly positive serum. The serum to be tested can be preserved with 0.4 per cent. carbolic acid without interfering with the reaction. In order to make the test they proceed as follows: To 9 c.c. of serum diluted 1 to 20 with saline is added 1 c.c. of distilled water and counted before and after incubating for one hour at 50° C. This is used as a control. To 9 c.c. of serum diluted 1 to 20 with saline is added 1 c.c. of antigen emulsion of the desired strength. This is counted only after incubation for one hour at 50° C. The fluid after being taken from the incubator should be cooled to room temperature very gradually. If done hastily the reaction is rendered worthless.

Izar,[19] writing on the properties of tumor lipoids (an experimental study), states that the sera of rats in which transplantation of rat sarcoma had failed were meiostagmine negative. That in a guinea-pig in which he had transplanted rat sarcoma, he was able to demonstrate a sarcoma meiostagmine as long as the tumor remained. As soon as it became completely absorbed the guinea-pig became meiostagmine negative. That the injection of rat sarcoma antigen into rats or guinea-pigs makes their sera meiostagmine positive.

A. Ascoli[20] tested the reaction in the foot and mouth disease of cattle, and found 22 of 28 cases positive, whereas, of 36 non-infected animals, only 2 gave an increase of more than 1 drop.

In November, 1910, Micheli and Cattoretti,[21] in attempting to verify the results of Ascoli, reported: In 27 malignant neoplasms the sera were "more or less" meiostagmine positive. In 4 cases the sera were distinctly negative. In typhoid their results were not in accord with Ascoli's findings. Sera of typhoid immune rabbits gave negative reactions. With syphilitic sera his results were positive against syphilitic antigen, but he also obtained positive results with tumor sera against syphilitic antigen. A great number of tuberculosis cases in his hands gave the reaction against tubercle antigen.

Kelling,[22] in January, 1911, in testing Ascoli's method, found in 45 cases of malignant growths positive results in but 21 (47 per cent.), even though he considers an increase of 1½ drops as a positive reaction.

During the Thirty-ninth Surgical Congress, held at Berlin in March, 1910, at which Ascoli spoke concerning his results, Stuber, of Berlin, reported that at Bier's clinic they were unable to obtain any results with the meiostagmine reaction in undoubted cases of malignant growth, even against imported Italian antigen. They had had similar negative results with antigen made from native animal tumors.

Other articles appeared during this time, but embodied nothing new, and are, therefore, only referred to in the tabulated literature.

In looking over the entire field covered by these workers, as recorded in the above literature, one cannot fail to observe the following facts:

Although the results obtained in the first article were all that could be desired, yet a radical change in technique was instituted in the reports that followed.

In the first article the serum antigen mixture was counted before and after incubation, while in the articles that followed the serum alone was counted, and the antigen having been added, the mixture was incubated and again counted. The increase in the number of drops ascertained by the difference in these counts was used for the diagnosis. We see no logical reason for this change; in fact, we consider the original technique only as correct from a serological standpoint.

Further, a discrepancy was noted in the preparation of syphilitic antigen. Izar[4] makes the statement that guinea-pig heart and normal human liver were worthless as antigen, whereas six weeks later he[6] states positively that they can be used very well as antigen, and gives no explanation why he changed his opinion.

Again, for no apparent reason, the diluent of the antigen is changed from normal saline to distilled water, while the diluent of the serum remains unchanged.

Izar[4] states that syphilitic antigen can be used in any dilution from 1 to 25 to 1 to 100,000—rather a remarkably wide range—and yet in a subsequent article he[6] draws special attention to the necessity of absolute titration. Again, while $\frac{n}{1000}$ acetic acid used as a diluent of sera is said by Ascoli[8] to increase the activity of antigen remarkably (2 to 5 drops), yet strange to say, this desirable modification is not employed in any of his subsequent work.

Although all the work done seems to point to the absolute specificity of the reaction in malignant disease, and in spite of the fact that Micheli and Cattoretti[11] found that not all malignant tumors were of value in antigen manufacture, yet Verson[12] was able to get positive results with colloid goitre antigen against sera of malignant disease.

Aside from these changes in technique, it is a matter of surprise to note the apparent lack of interest displayed in medical centres other than those of Italy in this simply performed reaction, which is of such vast importance clinically.

While these articles were appearing, we made tests in the pathological laboratory of Mount Sinai Hospital, introducing, as they appeared, the changes in technique brought out by the Italian investigators. We have never attained what we have considered to be a positive result. We submit the following protocols.

The first tests were made in an attempt to verify the reaction as a diagnostic aid in typhoid fever. As antigen, an extract of typhoid bacilli was prepared after the method of Neisser and Shiga.[2] The serum was obtained from 4 typhoid suspect cases:

		Agglutination.	
Case I.	E. F.	Widal negative.	No blood culture.
Case II.	S. R.	Widal positive.	No blood culture.
Case III.	F. G.	Widal positive.	Blood culture showed typhoid bacillus.
Case IV.	J. P.	Widal negative.	No blood culture.

Technique: Antigen, 1 to 500 dilution in 0.85 per cent. NaCl solution, 1 c.c. used. Serum, 1 to 10 dilution in 0.85 per cent. NaCl solution, 9 c.c. used. Incubated at 37° C. for two hours.

	Count before incubation.	Count after incubation.
Case I	46	47
Case II	46	46 + 10
Case III	46 + 2	46 + 15
Case IV	46 + 15	47 + 5
NaCl (control)	47 + 5	48 + 5

Following this a cross-test was made using a known typhoid serum, a known syphilitic serum, a typhoid antigen, and a syphilitic antigen. The known typhoid serum was from Case III of the previous list. The syphilitic serum gave a strongly positive Wassermann reaction. The typhoid antigen was that used in the previous experiment. The syphilitic antigen used was the acetone insoluble fraction obtained from a congenital syphilitic liver dissolved in methyl alcohol. Of this, 1 c.c. of a 1 to 25 dilution in normal saline was used.

	Before.	After.
Typhoid serum, 1 to 10, 9 c.c. 1. Typhoid antigen, 1 to 100, 1 c.c.	47 + 5	48 + 5
Typhoid serum, 1 to 10, 9 c.c. 2. Syphilitic antigen, 1 to 25, 1 c.c.	47 + 5	48 + 5
Syphilitic serum, 1 to 50, 9 c.c. 3. Syphilitic antigen, 1 to 25, 1 c.c.	47 + 5	47 + 5
Syphilitic serum, 1 to 50, 9 c.c. 4. Typhoid antigen, 1 to 100, 1 c.c.	47	46 + 5
5. NaCl, 0.85 per cent., 10 c.c.	45 + 5	46 + 5

The Reaction in Malignant Tumors. The antigen used was prepared according to the method set forth by Ascoli and Izar.[5] The material used consisted of: (1) A round-celled sarcoma of the foot, diagnosticated microscopically. This will be referred to as "Antigen Sarcoma A." (2) A carcinoma of the breast, diagnosticated microscopically. This will be referred to as "Antigen Carcinoma B."

The sera used were obtained from the following patients:

Case I.—L. R. Clinical diagnosis was sarcoma of femur. Autopsy showed an embolic aneurysm of the femoral artery with malignant endocarditis.

Case II.—A. S. Clinical diagnosis was carcinoma ventriculi, with liver metastases.

Case III.—I. K. Clinical diagnosis was carcinoma ventriculi. Exploratory laparotomy, which showed a large inoperable tumor of the stomach.

Case IV.—S. S. Neoplasm of lung. Fluid withdrawn from chest showed sarcoma cells. Patient developed spinal metastases.

These sera were tested against both Sarcoma A and Carcinoma B antigen, with the following results, the serum being diluted 1 to 20 and the antigen 1 to 500.

SARCOMA ANTIGEN A.

	Before.	After.
Case I	53	53 + 3
Case II	53 + 4	54 + 5
Case III	53 + 6	54 + 5
Case IV	53 + 2	54 + 5

CARCINOMA ANTIGEN B.

	Before.	After.
Case I	53 + 16	53 + 4
Case II	53 + 12	54 + 4
Case III	53 + 2	54 + 2
Case IV	53 + 5	54 + 1

Thus far no positive results were obtained in dilutions of antigen as recommended by Ascoli and his pupils, and inasmuch as the serum dilution 1 to 20 was a constant factor in all the experiments reported, we were forced to turn our attention to the antigen as the possible source of error. Granted that the preparation of the antigen was strictly according to the directions laid down, it became then only a question as to what strength one should exhibit in order to obtain satisfactory results.

Our next experiment was made to determine whether Sarcoma A antigen if properly diluted would react to the serum of a known sarcoma case. The serum used was from a case of round- and spindle-celled sarcoma of the femur, proved microscopically.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	55 + 5	57
	1 to 50	53 + 8	54 + 4
	1 to 100	52 + 16	53 + 16
	1 to 250	52 + 8	53 + 12
	1 to 500	53 + 7	53 + 8
	NaCl control	52 + 9	53 + 8

Incubated in water bath two hours at 37° C. Receptacles being covered to prevent evaporation.

Similarly to the above, Carcinoma B antigen was tested against the serum obtained from a patient suffering from carcinoma of the rectum, recurrent after operation, and proved microscopically to be an adenocarcinoma with involvement of the lymph nodes.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	54 + 8	55
	1 to 50	52 + 18	53 + 3
	1 to 100	53 + 2	53
	1 to 250	52 + 10	54
	1 to 500	53 + 3	52 + 18
	NaCl control	52 + 8	54 + 12

Having obtained no results with these antigens, other antigens were made.

Antigen Carcinoma C was obtained from a carcinoma mammæ medullare, and tested against the serum of a patient suffering from a carcinoma of the gall-bladder. The gall-bladder was only partially removed at the time of operation and the diagnosis substantiated microscopically.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation
Serum, 9 c.c. diluted, 1 to 20	1 to 10	51 + 13	52 + 8
	1 to 50	51 + 12	51 + 14
	1 to 100	51 + 12	52 + 16
	1 to 250	51 + 7	52 + 18
	1 to 500	51 + 5	53 + 3
	NaCl control	51 + 10	52 + 13

The next antigen was made from rat sarcoma, furnished through the kindness of Dr. Richard Weil, and labelled Sarcoma Antigen D, and tested against a known case of sarcoma of the femur proved microscopically.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	61 + 10	62 + 2
	1 to 100	51 + 2	52 + 2
	1 to 500	51 + 12	62

In the meantime Ascoli[18] had modified his preparation of antigen, and hoping to obtain results with this newer technique of antigen preparation, we proceeded with antigens E and F.

Antigen E was made from a carcinoma proved microscopically, and was tested against the serum of a patient clinically suffering with a carcinoma of the esophagus.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	60 + 10	63 + 16
	1 to 50	53 + 10	54
	1 to 100	52 + 11	53 + 10
	1 to 250	52 + 10	53 + 6
	1 to 500	52 + 15	53
	NaCl control	52 + 5	53 + 10

Incubated for one hour at 50° C. and allowed to cool to room temperature for thirty minutes before second count was made.

The same antigen was tried against the serum of a case of carcinoma mammæ, with the following results:

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	57 + 8	60
	1 to 50	52 + 5	54 + 10
	1 to 100	52	54
	1 to 250	52 + 8	53 + 14
	1 to 500	52	54
	NaCl control	52 + 4	54 + 10

The dilution 1 to 10 might on casual inspection appear to be a positive test, but the control forces us to disregard it.

Antigen F was prepared from a medullary carcinoma mammæ, according to the new method, and was tested against a case of carcinoma ventriculi, diagnosticated clinically and verified by exploratory laparotomy.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	67 + 17	62 + 17
	1 to 100	54	52 + 5
	NaCl control	51 + 14	52 + 8

Incubated at 50° C. for one hour and cooled to room temperature before the second count was made. Evaporation prevented.

The same antigen was tested against an inoperable carcinoma of the rectum on whom a colostomy had been done.

	Antigen, 1 c.c. in dilution of	Before incubation.	After incubation.
Serum, 9 c.c. diluted, 1 to 20	1 to 10	55 + 3	66 + 10
	1 to 100	52 + 18	52 + 5
	NaCl control	52	52

In the last few protocols the use of a strong antigen dilution seems to give marked differences before and after incubation, but these are to be disregarded, since all investigators agree that antigen when used in dilution less than 1 to 50 gives unreliable results and may react strongly positive with normal sera. From a study of the above tables we must conclude that the meiostagmine reaction in our hands has not proved satisfactory as a diagnostic aid.

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THE TREATMENT OF BRONCHIAL ASTHMA.

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It is not my purpose to enumerate a catalogue of drugs, but to review briefly those agents which have seemed of most value in a series of cases seen by my colleagues and myself in the Touro clinic in the last couple of years.

In some 7000 patients we have seen 31 cases of bronchial asthma. Of these 31, 8 were white males and 6 white females, a total of 14 whites; 8 were negro males and 9 negro females, a total of 17 negroes. As the clinic is very nearly evenly divided between whites and negroes (a recent summary showing about 45 per cent. white and 55 per cent. negroes) there is no apparent difference in the incidence between whites and negroes. The same is true of the